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OPERATING MANUAL

MODIFIED HENDERSON APPARATUS

RALPH W. KUEHNE

SEPTEMBER 1967

UNITED STATES ARMY MEDICAL UNIT

WALTER REED ARMY MEDICAL CENTER

FORT DETRICK, FREDERICK, MARYLAND

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OPERATING MANUAL

MODIFIED HENDERSON APPARATUS

RALPH W. KUEHNE

ANIMAL ASSESSMENT DIVISION

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13. ABSTRACT

The basic principles, utilization and operation of the modified Henderson apparatus are presented in this illustrated manual. Details of aerosol generation, animal exposure, sampling and dose determination are discussed, together with step-by-step operational procedures for aerosol exposures and decontamination. Included is a list of references describing studies undertaken with this apparatus and related sources of aerobiological information.

KEYWORDS:

Henderson apparatus
Aerobiology
Respiratory exposure
Aerosols
Air Sampling
Collison generator
Impingers
Dosimetry

DD FORM 1 NOV 65 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

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OPERATING MANUAL

MODIFIED HENDERSON APPARATUS

I. DESCRIPTION

The modified Henderson dynamic aerosol generating device[1] of the U. S. Army Medical Unit was constructed in 1957. This apparatus has been successfully used to expose mice,[2] guinea pigs,[2,3] rabbits, monkeys,[2,4-10,21] pigs, chimpanzees,[11] and humans[12-14] to aerosols of bacterial spores,[3,4,6] vegetative bacteria,[5,7-9,11-15] viruses,[4,8,10] and toxins.[10,21]

The principle of operation is as follows: a suspension or solution of bacteria, viruses, or toxin is aerosolized by means of a Collison generator.[17] This generator is driven by compressed air at a rate of 8 L/min; it disseminates 0.2 ml/min of suspension or solution, producing particles 80% of which are < 2 μ in diameter. The resulting aerosol, mixed with 20 ft^3/min of wet and dry air combined to give a desired relative humidity (RH), travels along an exposure tube at the rate of 7.3 ft/sec. This stainless steel tube is 8 ft long and 6 inches in diameter. At the exhaust end the air passes successively through a deepbed fiberglass filter, an electric incinerator (425 F) and finally through another series of deepbed filters. A simplified flow diagram, Figure 1, shows the path of air through the system.

It is possible to expose simultaneously 30 mice, 12 guinea pigs or 3 monkeys by utilizing 3 ports which are attached horizontally to the exposure tube. Air is drawn from the tube past the animal's head and collected for assay by means of air samplers such as all-glass impingers (AGI-30's) operated from house vacuum lines. This operation is shown diagrammatically in Figure 2. Figures 3 and 4 show the apparatus photographically. Note the guinea pig restraining device in the center of Figure 3, and 4 cans in holders at the middle port. Slit samplers and impingers are shown in operating positions.

The impinger fluid is assayed by means of plate counts in the case of bacteria, animal titrations with viruses, and fluorescence meter determinations when a fluorescein tracer is used. Since the air flow of the sampler and the time of sampling is known, the concentration per liter of the aerosol can be calculated. The dose can then be determined by multiplying the concentration per liter by the number of liters inhaled. (This latter value is derived from the tidal volume of the animal[20] and the length of exposure.)

The apparatus was originally designed to have an aerosol concentration reproducibility of ± 0.5 log, but desired dosages have routinely been accomplished within 0.1 to 0.2 logs.

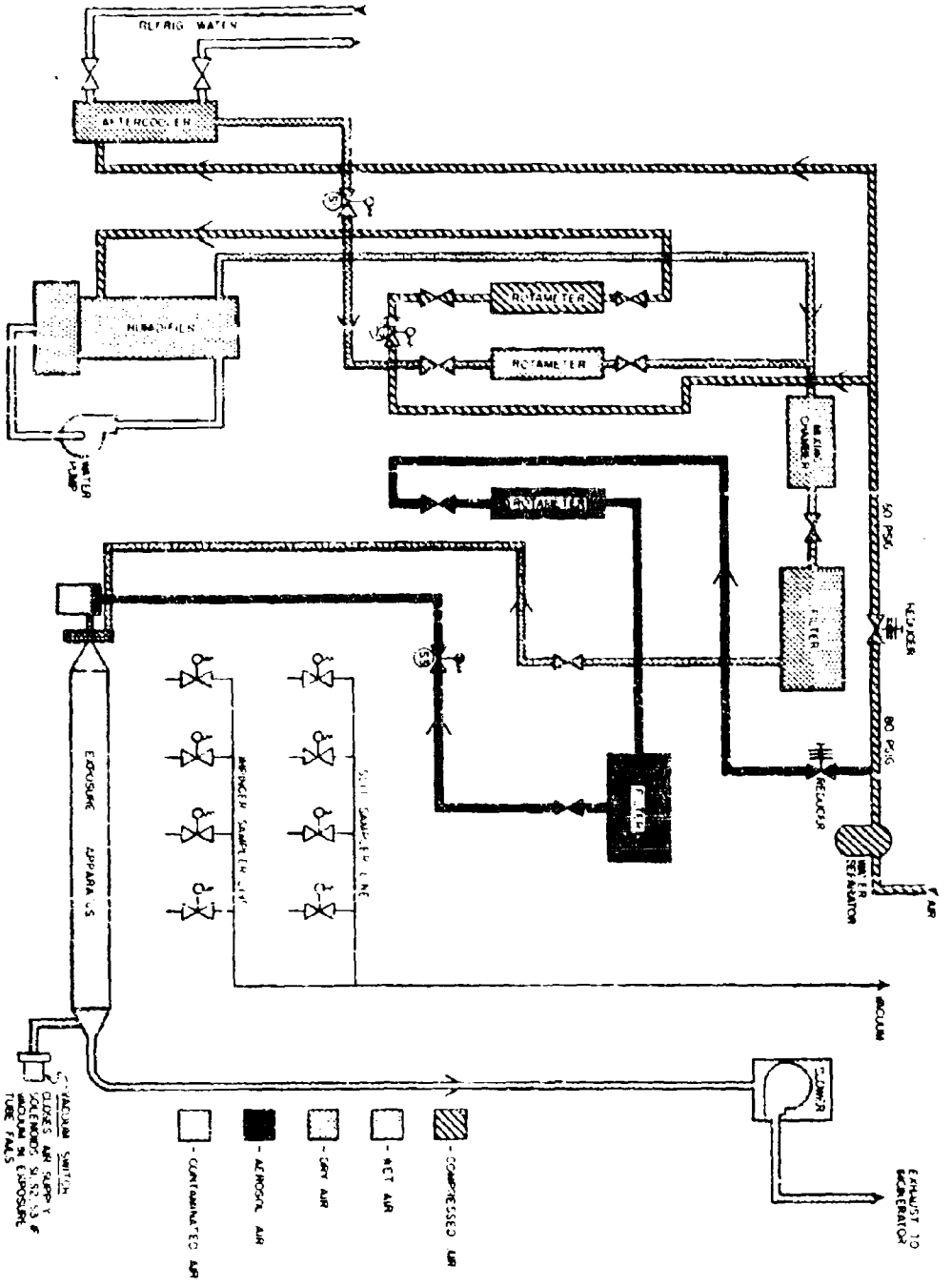


FIGURE 1. AIR FLOW DIAGRAM - AEROSOL EXPOSURE APPARATUS

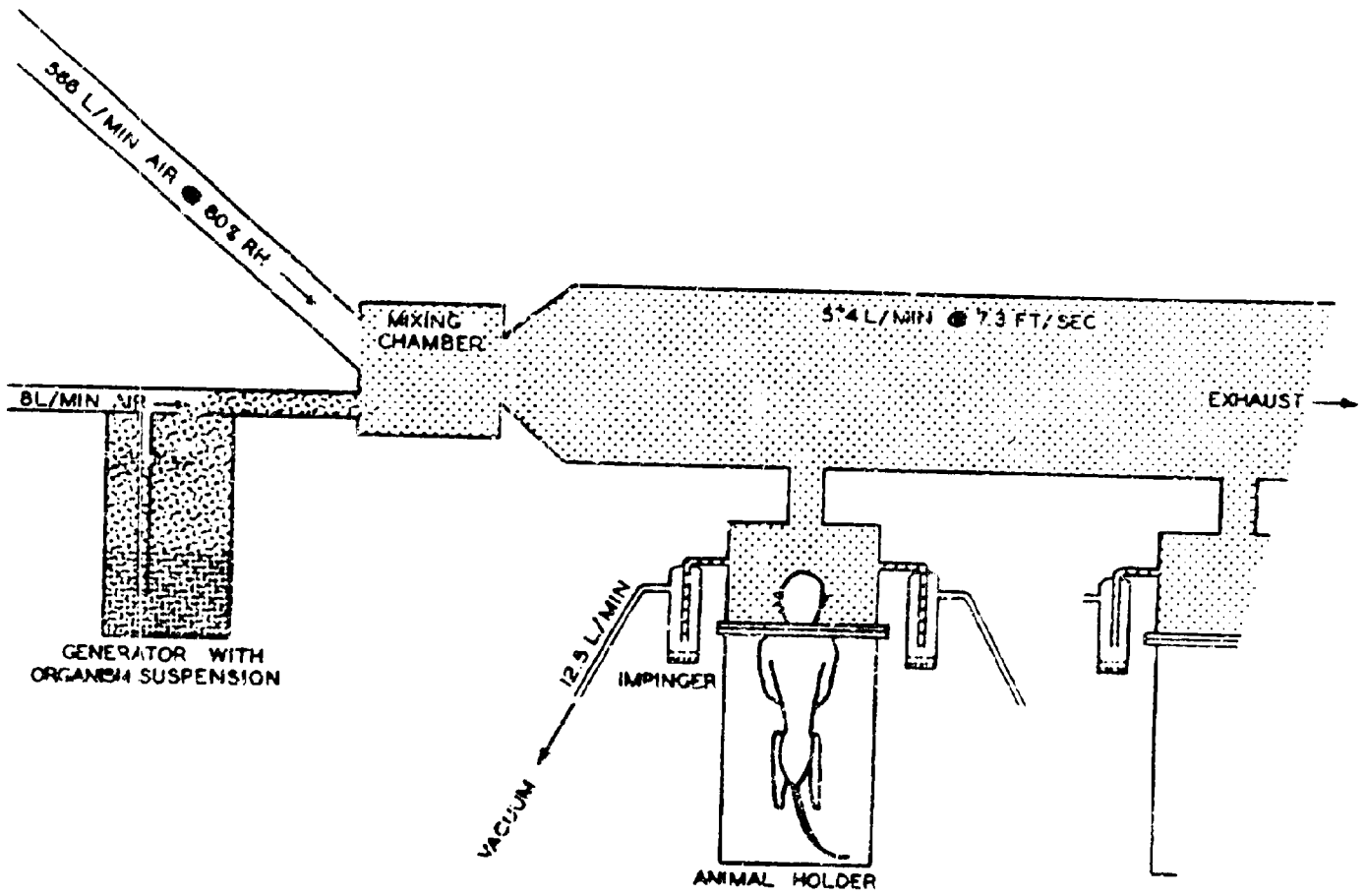


FIGURE 2. AEROSOL GENERATION & ANIMAL EXPOSURE.

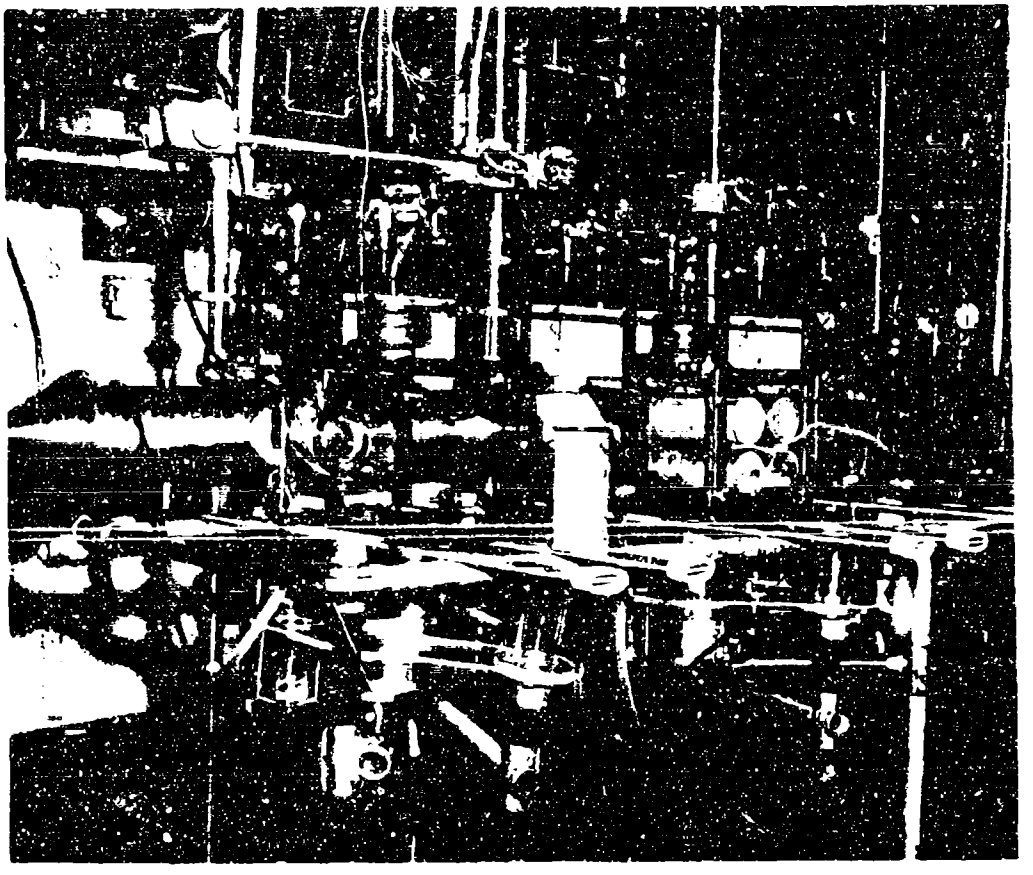


FIGURE 3. AEROSOL EXPOSURE APPARATUS (FRONT VIEW).

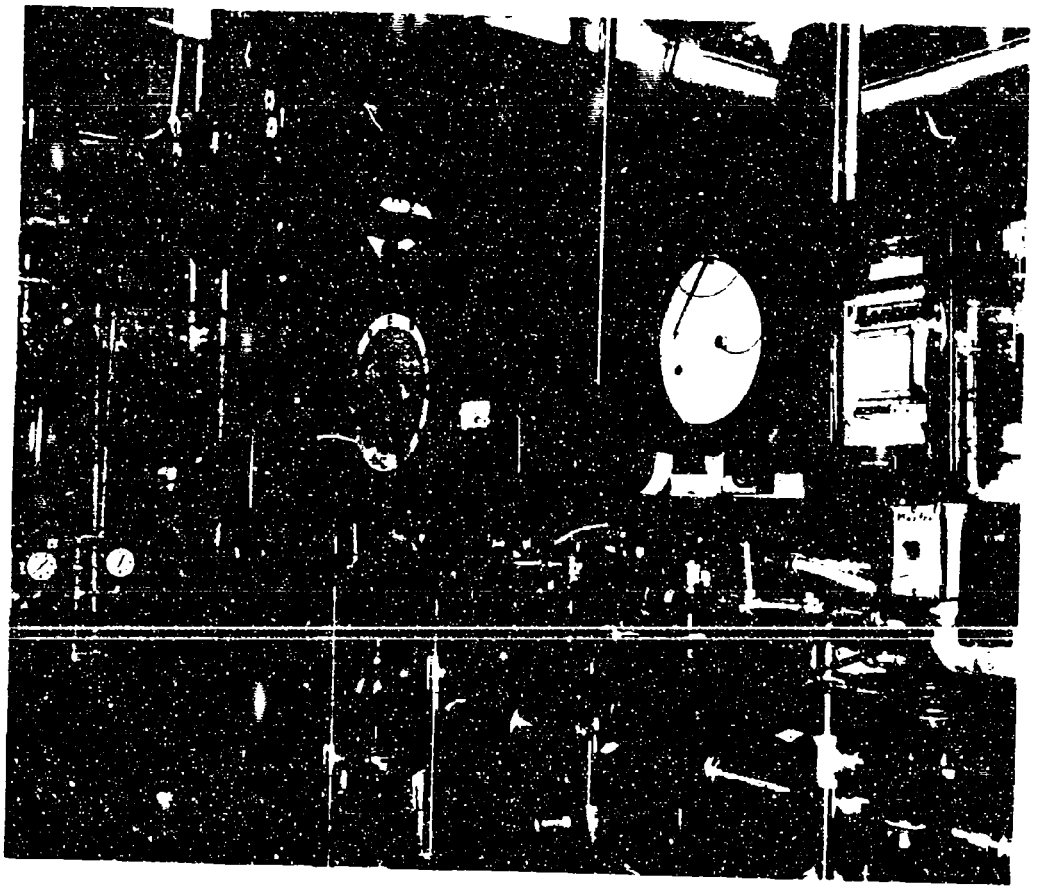


FIGURE 4. AEROSOL EXPOSURE APPARATUS (REAR VIEW).

The aerosol suite, shown in Figure 5, consists of the exposure room, an anteroom for storage and transfer of animals, and a shower and change room. This suite is kept at a 0.2 - 0.3-inch negative air pressure differential to the main hallway. A laboratory is located adjacent to the suite and connected by means of a pass box. This facilitates assay procedures since samples can be delivered from the suite to the laboratory safely and rapidly. The laboratory personnel can communicate directly with the suite through a speaking diaphragm without a break in the barrier system.

II. GENERATION

A. GENERATOR HEADS

The Collison generator is shown in Figure 6. Two heads shown in Figure 7 have been used with the Collison generator: a round-sided one which has always been used with one bottom screw removed and a hexagonal one which has been used only with all 3 bottom screws in place. The hexagonal head is used exclusively for toxin generation. The reason for removing one screw in the round-sided head is merely one of precedent, i.e., for some unknown reason all early calibrations were made in this manner and the practice has been continued. The heads can be autoclaved; side and bottom holes can be cleaned with syringe needle cleaning wires.

B. GENERATOR JARS

One pint, screw-topped jars are normally used as fill suspension containers. These can hold 100 ml of material which is usually sufficient for about 20 min spray time for human exposures. For monkey exposures, one fill can be used for 30-45 min spray time without much alteration of concentration.

Several modified jars have been used for special purposes. One is a shortened jar which requires only 75 ml of suspension material. When only a small amount of material is available, a modified well jar can be employed which utilizes only 20 ml. This jar can be used for about a 30-min spray time, although a higher concentration occurs with time due to the settling out of large particles.

C. JACKETS

When a standard screw-topped jar is used with living organisms, a water jacket (stainless steel) is clamped over the jar. (Figure 6) Prior to use this jacket is filled with water and kept at 4 C. When the well jar is used with living agents it is placed in a beaker containing ice water during operation. When nonviable agents are used the jar is placed in a constant temperature water bath set at any desired temperature.

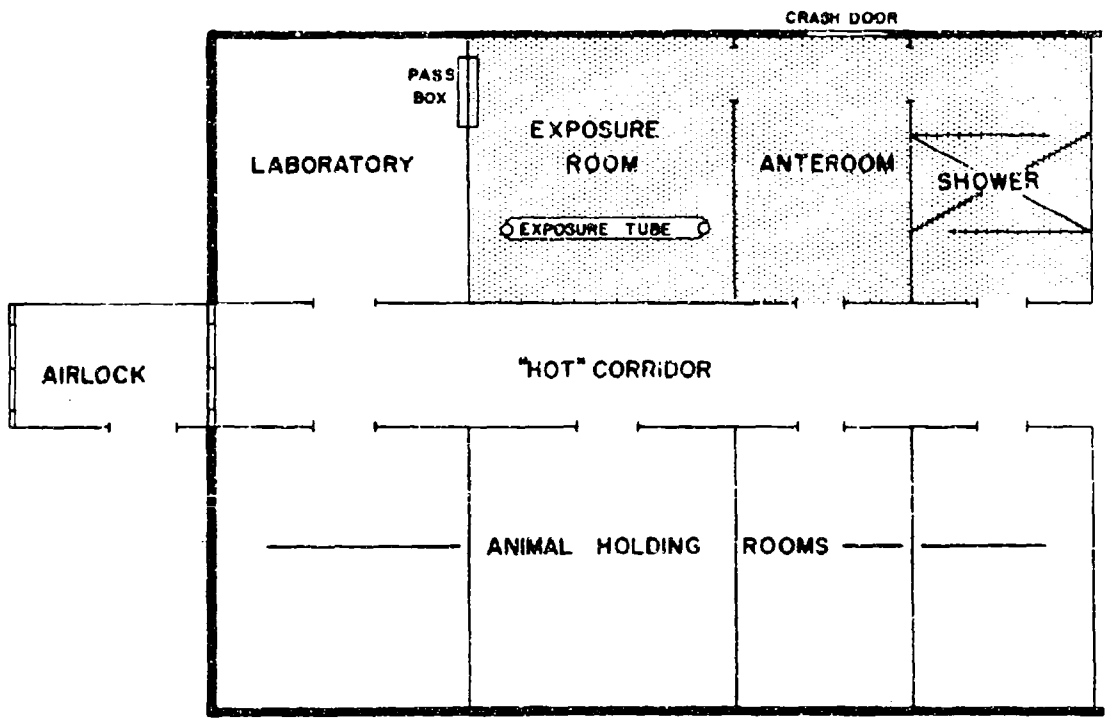


FIGURE 5. AEROSOL EXPOSURE SUITE AND SURROUNDING AREAS.

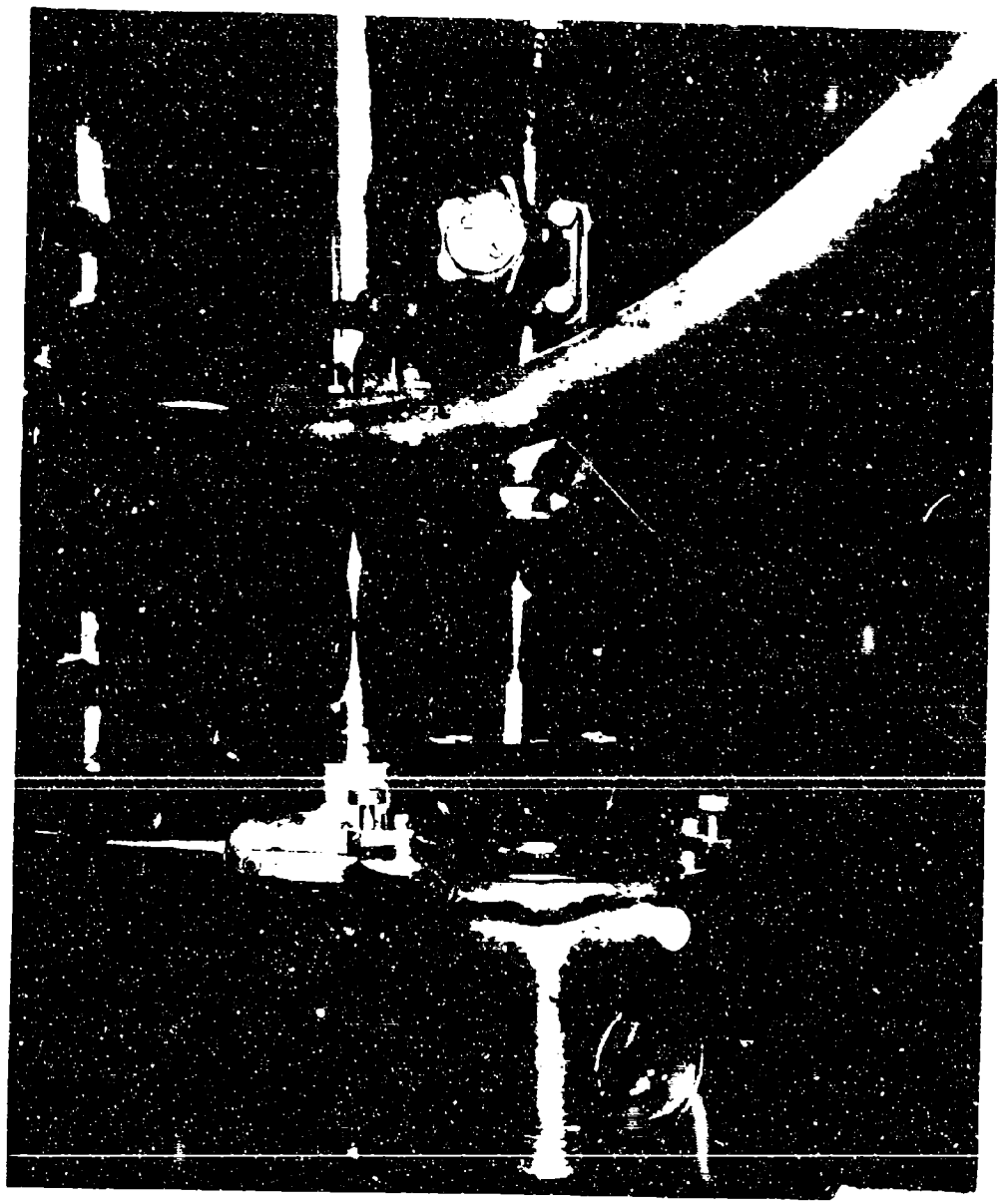


FIGURE 6. COLLISON GENERATOR WITH WATER JACKET.

FIGURE 7. HEXAGONAL AND ROUND COLLISON GENERATOR HEADS.

D. SPRAY FLUIDS

The following fluids are used as suspending fluids for various agents: phosphate buffered saline (PBS) solution, pH 7.4, containing 1 or 2% normal rabbit serum (NRS) for viruses; tryptose saline for Pasteurella tularensis; sterile distilled water (for injection) for Bacillus anthracis spores or staphylococcal enterotoxin B.

E. CULTURES

Bacterial, rickettsial and viral cultures and toxins must be obtained or prepared well in advance of the exposure date to allow sufficient time for determination of purity, virulence, and aerosol characteristics. Bacterial cultures must be plated to determine stock concentration and should be titrated parenterally in animals to determine virulence. Viral cultures should also be titrated in animals to determine concentration of stock material. B. anthracis spores should be heat shocked for 30 min at 60 C. All material to be aerosolized should be plated to assure absence of contamination and calibration trials should be conducted prior to actual exposure to determine percentage recovery of the aerosolized agent. Requests for cultures from outside agencies should state the type of study and animal species to be used, requesting cultures devoid of allergenic constituents.

III. COLLECTION

Many types of air samplers[18] can be utilized. Those usually employed are listed below:

A. IMPINGERS

This all-glass impinger (AGI-30), shown in Figure 8, is the most widely used for the collection of viruses, bacteria, and toxins. The sampler contains a 12.5-L/min critical orifice through which the air is drawn by means of a vacuum; the organisms are impinged in 20 ml of collecting fluid contained in the sampler. With this sampler a sustained vacuum of at least 15 inches of Hg is required to maintain the critical pressure ratio across the orifice, which, in turn, produces sonic velocity. Therefore, vacuum gauges have been installed in each vacuum line. The impingers can be checked for proper air flow by using a wet test meter. The length of the tubing connecting the impinger to the exposure port affects recovery and should be kept as short as possible.

Collecting fluids used in the impingers are the same as the spray suspension fluids for the respective agents (P. tularensis, tryptose saline; viruses, PBS + NRS; spores and enterotoxin, water for injection). When tryptose saline or phosphate buffer is used in the impinger, one drop of Dow-corning Antiform A (Dow-Corning Corp., Midland, Mich.) must

be added. Impingers (Ace Glass Co., Vineland, N. J.) are normally run for no longer than 10-15 min because of evaporation, concentration of media components, and deleterious effect of continued aeration on fragile organisms.

B. SLIT SAMPLERS

Fort Detrick slit samplers (Figure 9) have been used on occasion for low-concentration aerosols of bacteria and viruses.[16] In this sampler the aerosol is collected at a rate of 28.3 L/min; the organisms are impacted on a rotating 150 x 20 mm agar plate. With bacteria, this sampler renders a time-concentration relationship which requires no laboratory manipulation since the plate itself is incubated and the colonies counted. However, this sampler gives the number of particles, not necessarily the number of organisms. The sampler contains no critical orifice so it must be precalibrated with a wet test meter and a manometer board which is marked when a flow of 28.3 L/min is attained. By the use of a valve, the sampler flow is thereafter set at this mark during sampling. The agar concentration in the plates should be 1.5 - 2.0% to avoid damage during sampling. For viruses, PBS + NRS + 8.0% gelatin has been used. After sampling, the plate is liquefied at 37°C for 30 min and titrated in animals.

C. MEMBRANE FILTERS

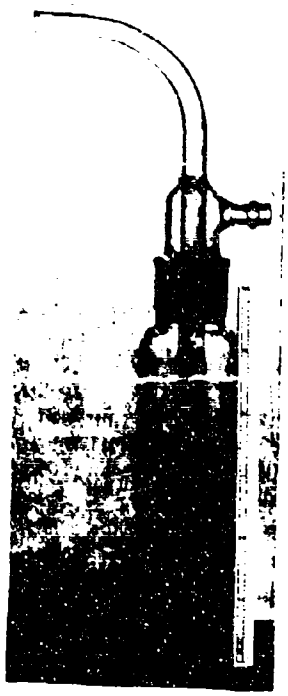
Membrane filters (Figure 10) can be used for collection of spores, toxin, or dye. Millipore filters (0.3μ) (Millipore Filter Corp., Watertown, Mass.) have been used effectively to recover aerosolized sodium fluorescein. When spores are collected the filter can be washed or, in the case of low concentration, placed directly on agar, incubated, and counted directly. Toxin or dye can be washed off with water. Since this sampler has no critical orifice, the flow rate must be measured or a critical orifice can be inserted behind the sampler, provided the flow rate in the system is then determined.

D. OTHER SAMPLERS

Samplers such as Cascade impactors (Figures 11 and 12,, in which organisms are impacted on glass slides, have been used for special purposes, e.g., fluorescent antibody studies.[15] However, it is usually necessary to employ AGI-30's at the same time to give an accurate estimate of aerosol concentration. When Cascade impactors are used, an in-line 18 L/min critical orifice is used.

IV. ASSAY

Although impinger samples have been held up to 2 hr under refrigeration without significant change in viability, they should be processed as soon as possible after collection. Some organisms (e.g., spores) can be held > 24 hr under refrigeration in the frozen state. After sampling, the amount of remaining collecting fluid should be determined volumetrically, or if greater accuracy is desired, by weighing the sampler before and after use.



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SAMPLERS

FIGURE 8. ALL-GLASS IMPINGER (AGI-30).
FIGURE 9. FORT DETRICK SLIT.
FIGURE 10. IN-LINE MEMBRANE FILTER.

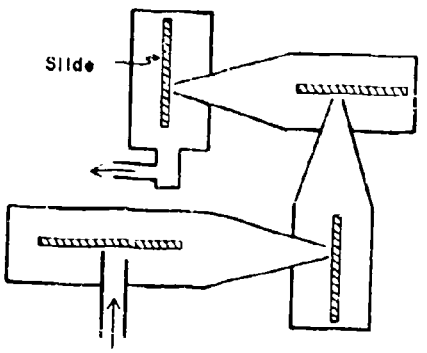

FIGURE 11 & 12. 4-STAGE CASCADE IMPACTOR (DIAGRAM & PHOTOGRAPH).

A. BACTERIA

Serial log dilutions are made in appropriate diluting fluid, e.g., sterile distilled water for spores, tryptose saline for P. tularensis; 0.2 ml of each dilution should be streaked on a minimum of 3 plates. The countable plate range is considered to be 30-300 colonies/plate. The average plate count from the appropriate dilution level is then used to compute the concentration of organisms in the impinger fluid. B. anthracis spores are plated on nutrient agar and counted after incubation for 24 hr at 37°C. P. tularensis is plated on glucose cysteine blood agar (GCBA) and incubated at 37°C for 48-72 hr before counting. Dilutions of the impinger fluids can also be inoculated into animals to determine virulence.

B. VIRUSES

Serial log dilutions of viruses are made in appropriate diluting fluid, e.g., PBS + NRS. Portions of each dilution are then inoculated into appropriate animals (6 animals/dilution) which are observed for reaction. Values for median lethal or median immunizing doses (LD_{50} or ID_{50}) are calculated by the method of Reed and Muench;[19] the virus concentration of the impinger fluid may then be determined. When viral vaccines are aerosolized, animals must be bled for serology and/or challenged with virulent virus at a later date. The aerosol concentration is then determined and expressed as ID_{50}/L.

C. TOXINS

When toxins are used the impinger fluid can be diluted and inoculated into animals and/or subjected to protein or total nitrogen analysis. If a dye tracer such as sodium fluorescein has been added, the dye is measured directly with a Photovolt fluorescence meter (Photovolt Corp., New York, N. Y.) by which the concentration/L of dye can be calculated. This value multiplied by the toxin:dye ratio indicates the concentration of toxin per liter.

V. CALCULATIONS

A. CLOUD CONCENTRATION

To estimate the number of organisms or infectious units/liter of aerosol, the sampler fluid must first be quantitated as above (Section IV. A, B or C). A general formula for estimating Concentration/L of Aerosol is:

$$(1)\quad \frac{(\text{Concentration/ml sampler fluid}) \times \text{Sampler volume}}{\text{Sampler air flow rate} \times \text{Sampling time}}$$

With bacteria, if 0.2 ml is plated, the formula becomes:

$$(1a)\quad \frac{(\text{Average count}/0.2\text{ ml} \times 5 \times \text{Dilution factor}) \times \text{Sampler volume}}{\text{Sampler flow rate} \times \text{Sampling time}}$$

With viruses, if 0.5 ml inoculum is used:

$$(1b)\quad \frac{(ID_{50} \text{ or } LD_{50}/0.5\text{ ml} \times 2 \times \text{Dilution factor}) \times \text{Sampler volume}}{\text{Sampler flow rate} \times \text{Sampling time}}$$

With fluorescein-toxin suspension, to obtain toxin concentration:

$$(1c)\quad \frac{(\text{Concentration of fluorescein/ml impinger fluid}) \times \text{Sampler volume} \times \text{toxin:fluorescein ratio}}{\text{Sampler flow rate} \times \text{Sampling time}}$$

B. DOSE DETERMINATION

Estimation of animal respiratory dose is dependent on the number of viable organisms/L of air, respiratory tidal volume of the animal, and duration of exposure. The formula for Inhaled Dose is:

(2) Concentration of aerosol/liter at exposure time X
Minute respiratory volume (breathing rate) of animal X
Exposure duration

The breathing volume of animals is usually obtained by Guyton's formula:[20]

(3) Breathing rate (ml air/min) = $2.10 \times (\text{Body weight in gm})^{3/4}$
= 0.32 ml/gm per min

The respiratory volumes commonly used are:

Rhesus monkey (3 kg), 1 L/min
Guinea pig (300-350 gm), 0.1 L/min
Mouse, 0.025 L/min

Volunteer exposures involve measuring the expired air directly with a dry gas meter connected to the mask with a 2-way valve system. Volunteers usually breath about 10 L of air in their 1-min exposure period.

C. SPRAY FACTOR

The spray factor (SF) is the ratio of the number of organisms in the spray suspension to the number of organisms in the aerosol, expressed logarithmically, i.e.,

$$(4)\quad SF = \frac{\text{Concentration/ml of suspension}}{\text{Concentration/L of aerosol}}$$

A sample calculation would be: $SF = \frac{6 \times 10^9}{2 \times 10^5} = \frac{10^{9.778}}{10^{5.301}} = 4.477$

A spray factor must be determined for each type of suspension fluid, each time the spray head or air flow is changed, each time the apparatus is dismantled and reassembled, and with each new culture. The spray factor is used in determining the concentration of organisms required in the suspension to effect the desired dose.

(5) Concentration/ml Suspension = SF X Concentration/L Aerosol

D. EXAMPLE OF DETERMINATIONS REQUIRED FOR AN EXPOSURE

Assume that monkeys are to be exposed to 10,000 cells of F. tularensis during a 5-min exposure period.

1. Stock Culture Concentration

Initial plating of stock culture might give the following results: (0.2 ml/plate on each of 3 plates)

10^{-7} plates = TNTC
10^{-8} plates = 93, 86, 85: Average = 88.0/0.2 ml or 440/ml

(Since the 10^{-8} plates are in the 30 to 300 range, this average is used.)

440×10^8/ml = 4.4×10^{10}/ml

2. Calibration Run

A fill suspension is made of a 10^{-3} dilution of the stock culture. This should be equal to about 4.4×10^7/ml. The fill is then used to spray 2 clouds of 5-min duration each. Each cloud is sampled with 2 impingers (pooled after sampling) located at each of the 3 monkey ports. The following plate counts might be attained:

Sample	Dilution	Count/0.2 ml	Average
Fill suspension	10^{-5}	79, 83, 103	88.33
Cloud #1, Port A	10^{-1}	90, 110, 85	95.00
Cloud #1, Port B	10^{-1}	83, 94, 76	81.00
Cloud #1, Port C	10^{-1}	109, 86, 89	94.67
Cloud #2, Port A	10^{-1}	87, 94, 99	93.33
Cloud #2, Port B	10^{-1}	106, 106, 94	102.00
Cloud #2, Port C	10^{-1}	140, 107, 115	120.67

Assuming impinger volume of 20 ml and air flow of 12.5 L/min, and using Equation (1a); Section V. A.:

$$\text{Conc/L of aerosol} = \frac{\text{Total number of organisms in impinger}}{\text{Number of liters sampled}} \quad \text{(Equation 1)}$$

$$= \frac{(\text{Count/0.2 ml} \times 5 \times \text{Dilution factor}) \times 20 \text{ ml}}{(12.5 \text{ L/min} \times 5 \text{ min})}$$

$$= \frac{\text{Average count/0.2 ml} \times \text{Dilution factor} \times 100}{62.5}$$

$$= (\text{Count/0.2 ml} \times \text{Dilution factor}) \times 1.6$$

$\therefore$ Cloud #1, Port A $= 95.00 \times 10^1 \times 1.6$

$= 1520$

$\log 1520 = 3.18184$

Thus, the following per-liter recoveries are determined:

Cloud #1, Port A	= 1520	log = 3.18184
Cloud #1, Port B	= 1296	3.11261
Cloud #1, Port C	= 1515	3.18041
Cloud #2, Port A	= 1493	3.17406
Cloud #2, Port B	= 1632	3.21272
Cloud #2, Port C	= 1931	3.28578

Since the fill suspension equalled 4.42×10^7/ml (88.33/0.2 ml $\times$ 5×10^5 dilution factor), expressed logarithmically as 7.64542, and the

$$SF = \frac{\text{Concentration/ml of suspension}}{\text{Concentration/L of aerosol}}$$

, each of the log per-liter recoveries are subtracted from 7.64542 to give 4.46, 4.53, 4.47, 4.47, 4.43, and 4.36, or an average spray factor of 4.45.

3. Fill Concentration

Since the spray factor has now been determined (4.45), and the count of the stock culture is known (4.4×10^{10}/ml), the fill concentration necessary to give the desired dose (10,000 organisms) can now be determined.

Since the tidal volume of rhesus monkeys is 1 L/min, during a 5-min exposure they will inhale 5 L of aerosol. Therefore, to receive 10,000 organisms the aerosol concentration must equal 2,000 organisms/L (log 3.30103). Since Concentration/ml suspension = Concentration/L aerosol (3.30103) X spray factor (4.45), this fill material should contain 7.75103 logs/ml, or 5.63×10^7 organisms/ml.

To attain this concentration, 1.28 ml of stock material (4.4×10^{10}/ml) are added to 8.72 ml tryptose saline to give a concantration of 5.63×10^{10}/10 ml, or 5.63×10^{9}/ml. Two further log dilutions are made to give the desired quantity which will contain 5.63×10^{7}/ml, e.g., 2 ml into 18 ml, followed by 10 ml into 90 ml will give 100 ml containing 5.63×10^{7}/ml.

To determine proper plating dilutions, if the aerosol concentration/L = 2,000, and Concentration/L = Count/0.2 ml X Dilution X 1.6, then count/0.2 ml X Dilution = $\frac{2000}{1.6}$, or 1250. Therefore, 0.2 ml of undiluted impinger fluid will contain 1250 organisms, 0.2 ml of a 10^{-1} dilution will contain 125, and 0.2 ml of a 10^{-2} dilution will contain 12.5. Since 125 organisms will produce countable plates, 0.2 ml of the 10^{-1} dilution should be plated as well as the dilution on either side (10^{0} and 10^{-2}), using 3 plates/dilution.

E. VIRUSES

When viruses are used, the spray factor is determined in the same manner using 50% endpoints based on animal titrations. The LD_{50} or LD_{50}/ml required in the fill suspension to give the desired dose is then calculated.

F. TOXINS

When a dye tracer is employed with a soluble toxin, a spray factor is determined by dividing concentration/ml of dye in the fill by concentration of dye/L of aerosol. The spray factor is then used to determine how much agent must be present in 1 ml of the fill to give the desired dose. Enough dye must be used in the fill to permit an accurate assay of impinger fluid, dependent on the range of the fluorescence meter (0.1-1.0 µg/ml gives most accurate results with the Photovolt meter). The dye concentration per liter of aerosol is multiplied by the dye:toxin ratio to give the toxin concentration.

VI. OPERATION

A. PREOPERATION CHECK

Before an exposure or calibration run is begun, it is necessary to check the following items:

1. Humidity recorder - turn on and check for proper operation. If indicator will not stabilize, replace dry cell battery. Also replace paper chart, if necessary, and check ink level in pen reservoir.

2. Drain mask air lines, if hoods or suits are to be worn. Bleed lines for 5 min or longer to exhaust any condensation and confirm that lines are not plugged.

3. Remove screw plug from humidifier and fill to top with water.

4. Drain air mixing chamber located above rotameters by opening valve until all water is exhausted.

5. Check vacuum pressure gauge located above sealed door. Gauge should read 20-25 inches Hg.

6. Check secondary air pressure. Gauge should read 55-60 psi.

7. Check primary air pressure. Gauge should read 55-60 psi.

8. Release tubing from manometer above control panel to make sure oil level reads 0. If not, adjust or add red oil (sp. gr. 0.086). Replace tubing.

9. Open trap door in hallway, close all doors to aerosol area, and make sure manometer on sealed door indicates at least 0.2 inches H_2O negative pressure in aerosol exposure suite.

B. OPERATION

1. Throw main switch on main electrical panel to ON position.

2. Turn exhaust blower switch, humidifier pump switch, and heater switch located below main electrical panel to ON.

3. Turn incinerator switch next to main panel to ON.

4. Turn vacuum blower switch, wet air switch, dry air switch and alarm switch located on control panel above tube to ON.

5. Turn humidity indicator and recorder to ON. Stabilize recorder (as per instructions located inside cover) and adjust indicator.

6. Set heater thermostat at 80°F.

7. Press secondary air ON button located to left of rotameters.

8. Open bottom wet air rotameter slowly to full open. Slowly open top valve and bring top of float to desired setting. Repeat with dry air rotameter. Settings will depend on air flow and relative humidity (RH) desired. A total reading of 200 on both rotameters will give about 20 ft^3/min, which is used for all viable agent exposures. The ratio of wet to dry air can be varied to attain the desired RH, but the total reading of 200 should not be exceeded. If tube shuts down during this operation, leaks or open port valves should be suspected. Also make sure primary air valve is closed at generator. When this air flow is used, vacuum manometer should read 0.2-0.3 inches H_2O. In cases where a lower spray factor is desired, e.g., when

using staphylococcal enterotoxin B, a total reading as low as 35 (3.5 ft^3/min) can be used, provided the large red exhaust wheel at the end of the tube is closed proportionately to give a vacuum manometer reading of 0.5 inches H_2O.

9. Stabilize 1 hr or more, making necessary adjustments in wet to dry air ratio to achieve desired RH.

10. When desired RH is attained, screw on fill jar containing suspension and open primary air valve at generator.

11. Place animals at ports as detailed in next section and attach impingers.

12. Turn switches to ON for respective impinger vacuum lines being used, leaving "main" switch OFF. Make sure port valves are open.

13. Set timer for desired spray time.

14. When ready to expose, turn "main impinger" and "aerosol" switches to ON. Check impingers for proper vacuum gauge reading and evidence of bubbling.

15. At the end of each exposure period (when timer buzzer sounds) turn "main" impinger and "aerosol" switches to OFF; close port valves before removing animals and impingers.

16. Five minutes before termination of the last exposure, turn incinerator off.

17. When last exposure is terminated, turn humidifier pump, heater, and humidity indicator to OFF.

18. Close top rotameter valves first, then bottom valves.

19. Remove fill jar, replace with jar of distilled water, set timer for 5 min and turn "aerosol" switch to ON. Allow to spray for this period, then turn "aerosol" switch to OFF.

20. Throw main switch on main electrical panel to OFF.

C. EXPOSURES

1. Monkeys

Monkeys are placed in inner metal boxes with the hinged end closed over the neck and fastened. The box is then placed in the outer aluminum box which is sealed by closing the attached fasteners. (Figure 13) Both boxes are placed on the support track which has previously been attached to the tube supports; connectio is made with the push-pull

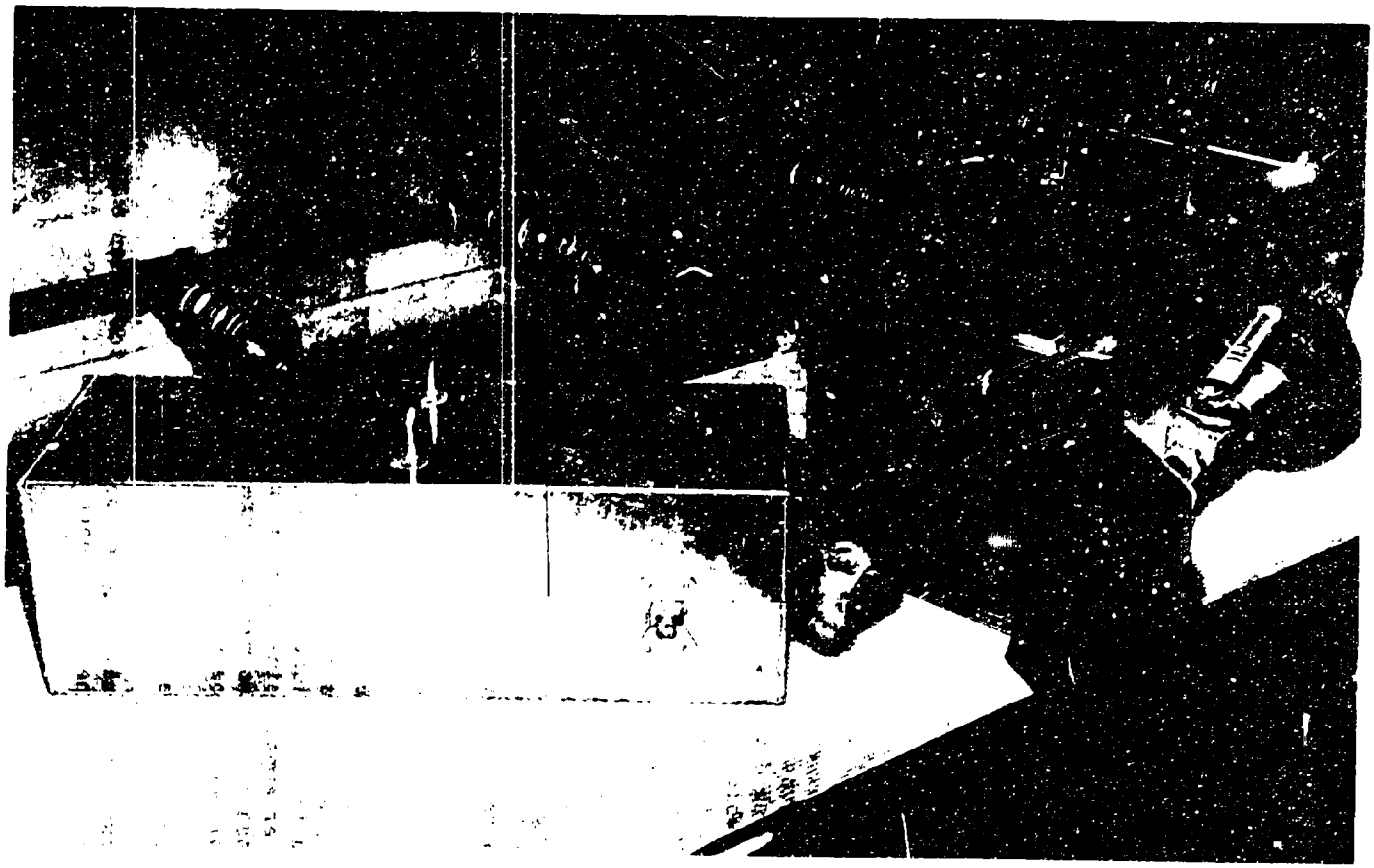

FIGURE 13. MONKEY-EXPOSURE BOX (SEPARATED).

valve which has been attached to each port, as shown in Figure 14. At this time the solenoid port valve should be in a closed position and the cannister valve attached to the outer box should be open, permitting the monkey to breathe filtered room air. The impingers are then attached to the outer box and vacuum lines connected. When exposure is to begin the cannister valve is closed, the port valves are opened, and the aerosol generator is turned on, permitting the monkey to breathe the aerosol cloud. When exposure is terminated, the port valves are closed, the impingers are removed, and the cannister is opened. The box is removed via the push-pull connector and taken to the aerosol suite anteroom, where it is connected to the vacuum manifold. The box is then airwashed for 10-15 min (90-100 L air/min), after which the box is taken to the animal holding room, the inner box is removed, and the monkey is put in a holding cage.

Normal monkey exposure time is 10 min, although times ranging from 2-20 min have been used. When anthrax spores are used for challenge, heads are shaven prior to exposure to reduce contamination during the holding period.

2. Guinea Pigs

For exposure of guinea pigs the plastic port boxes are used with manual port valves. Guinea pigs are placed in metal cans with hinged neck-restraining devices. Four guinea pigs are placed in each plastic holding rack which is then inserted into the port box and sealed by means of wing nuts. (Figure 3) Impingers are connected to the side of the boxes, being supported in impinger racks which have been previously attached to the tube supports. Manual port valves are opened prior to exposure. After exposure, the generator is stopped and the impingers are allowed to operate an additional 15 sec in order to evacuate contaminated air space within the box. The impingers are stopped, the manual valve is closed, and the impingers are removed. After removal of the wing nuts, the guinea pig holding rack is removed from the outer box, and the animals are returned to holding pens. The normal exposure time for guinea pigs is 10 min.

3. Mice

The exposure of mice is similar to that of guinea pigs except that 10 are placed in a rectangular covered wire basket with one basket being placed in each plastic port box. The box is sealed with a plastic cover plate or empty guinea pig holding rack. Exposures then progress as with guinea pigs. Normal exposure time is 10 min. (When mice are used, a total body exposure results.)

4. Human Exposures

An exposure panel (Figure 15) is used for human exposures. This panel is attached to the center port by means of a hose adapter, no

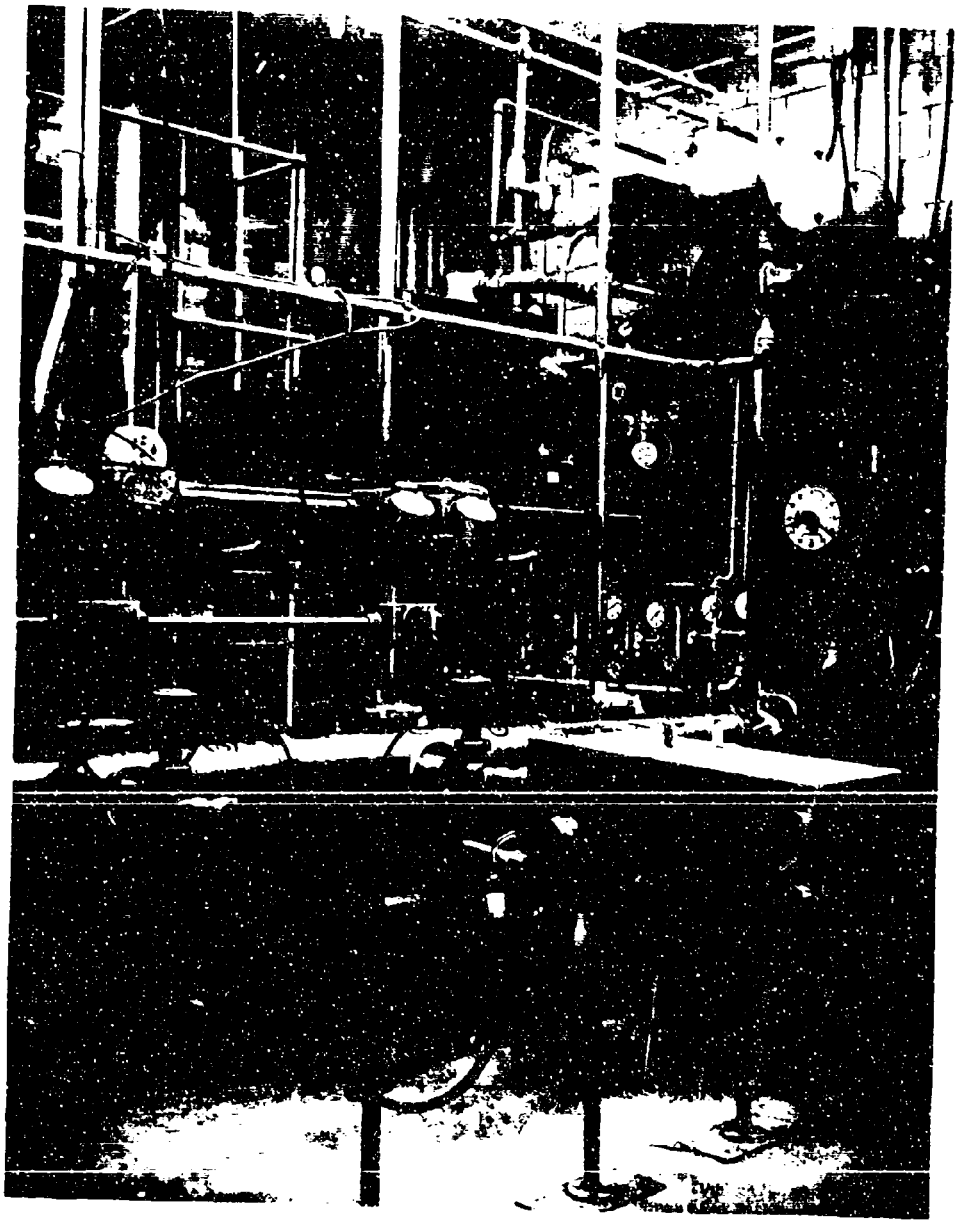

FIGURE 14. MONKEY- EXPOSURE BOX IN POSITION.

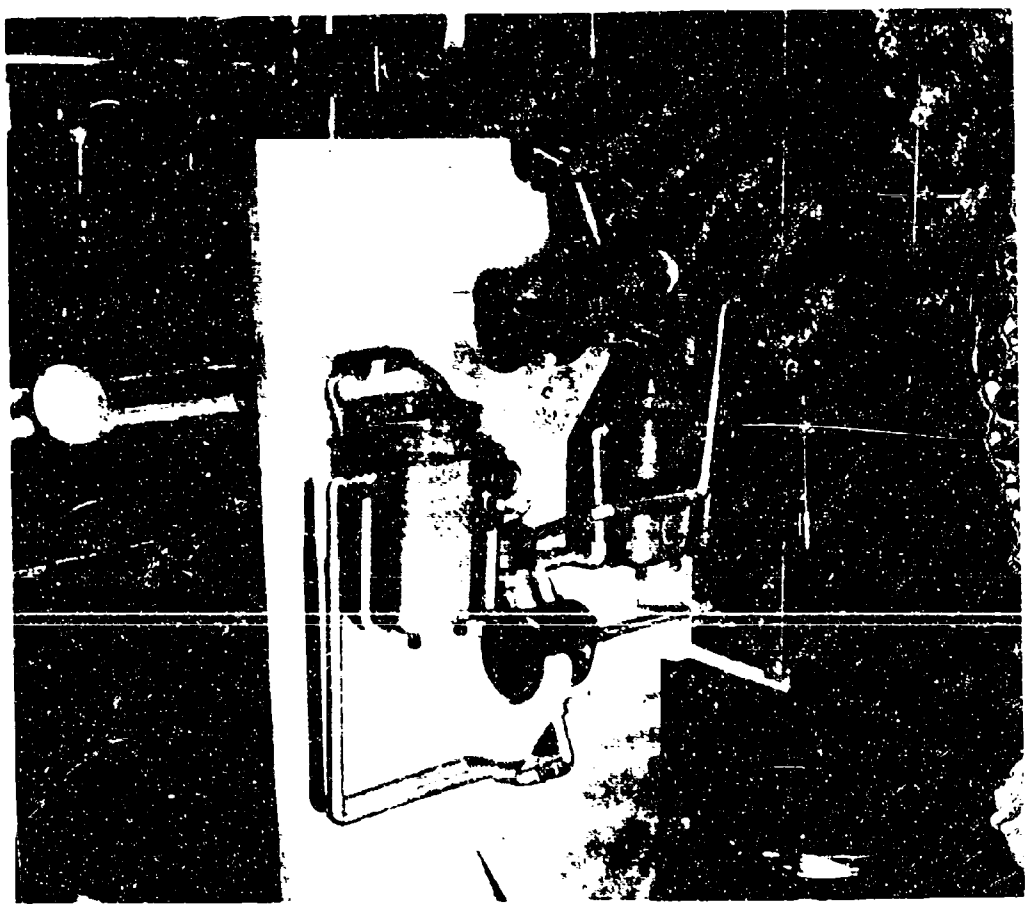

FIGURE 15. VOLUNTEER EXPOSURE PANEL

internal valve being used. The electrical timer is plugged into the nearest 110-V outlet. When the exposure level is in the down position, the subject breathes filtered room air which is exhausted into the dry gas meter at the right side of the panel. When the valve is moved to the up position, the timer is activated and the subject breathes air directly from the exposure tube exhaling into the left side dry gas meter. Two impingers are set in a holding rack which has previously been attached to tube supports on the back of the exposure tube. Tubing attached to the sampling adaptors nearest the point of exposure at the top and bottom of the tube is connected to the impinger inlets. Impingers are usually operated for 5 or 10 min during each exposure period. When the subject has established a constant breathing rate, as determined by the attending physician, the aerosol generator and impingers are turned on. At the discretion of the physician the subject is exposed during the sampling period for 90 sec, or 10 L inhaled, or 16 breathing cycles, whichever is first attained. (Extensive sampling has shown there are no statistical differences between any 1-min sampling intervals during a 10-min period.) Figure 16 shows a man in position for exposure.

5. Other Species

When exposure of other species of animals is contemplated, the holding device used depends upon the ingenuity of the operator. Exposure of swine, rabbits, and chimpanzees has been successfully accomplished using improvised equipment.

VII. PROTECTIVE EQUIPMENT

The exposure apparatus normally operates at an internal vacuum of 0.5-0.8 inches of H_2O. If the vacuum should ever drop below 0.2 inches, the primary and secondary air are automatically shut off by means of solenoid valves as a safety precaution. In spite of this, some respiratory protection is usually required for the operator. Rubber gloves and a surgical gown should be worn during any exposure unless the operator uses the ventilated suit. During aerosolization of most vegetative bacteria a respirator (e.g., American Optical type) is acceptable. When viruses or toxins are employed, the ventilated hood connected to a mask air line is required. The wearing of ventilated suits (Figure 17) is essential when spraying suspensions of pathogenic spores, after which the operator must go through the shower located in the suite. A tank of breathing air is available in the exposure room for emergency use.

VIII. DECONTAMINATION

A. EXPOSURE APPARATUS

The exposure apparatus can be completely disassembled and autoclaved or sterilized with ethylene oxide. All metal parts can be autoclaved, whereas, rubber or plastic compounds should be gas sterilized, as should

FIGURE 16. VOLUNTEER IN EXPOSURE POSITION.

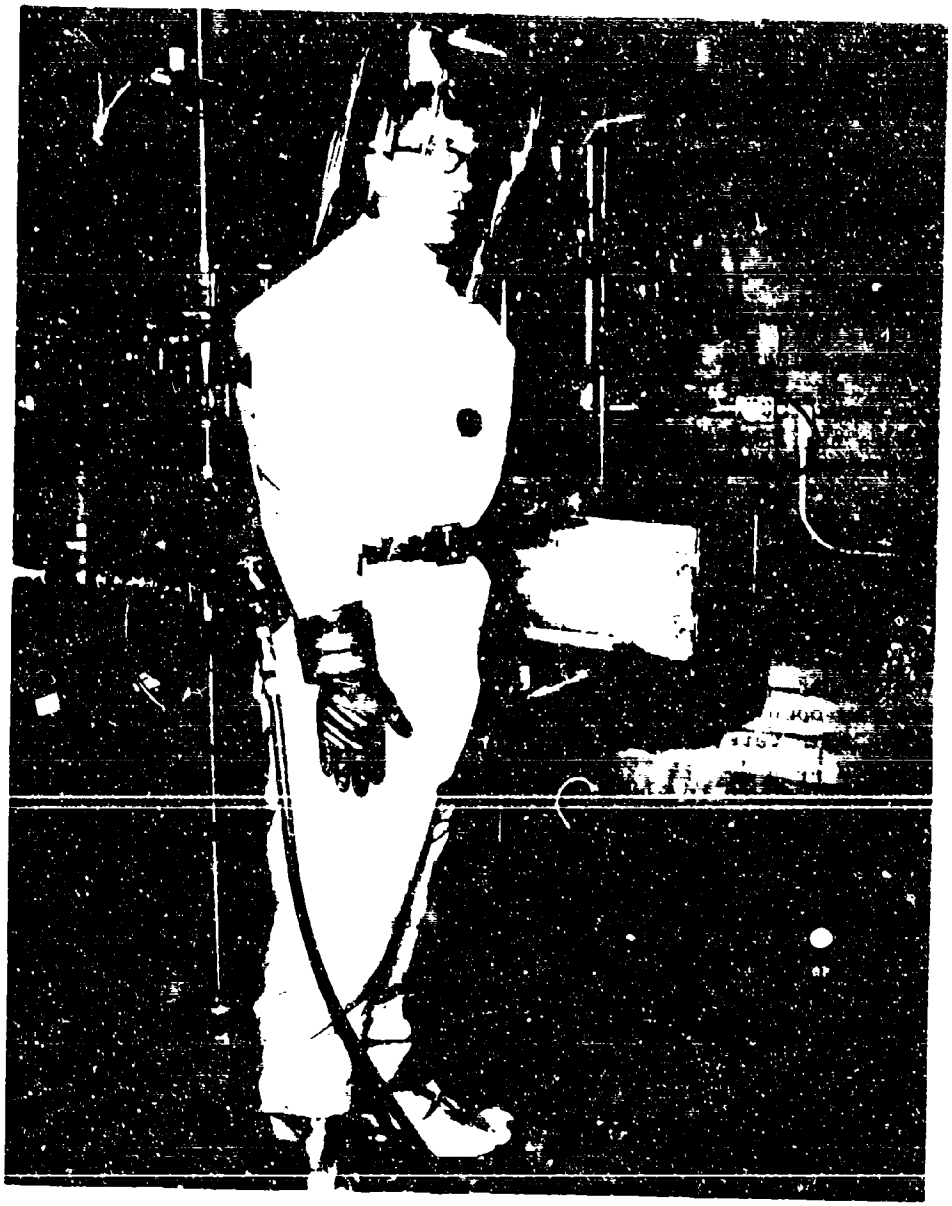

FIGURE 17. OPERATOR WEARING VENTILATED SUIT.

the humidity sensing element. All contaminated glassware and equipment should be autoclaved, or in the case of toxin, inactivated with 10 ppm sodium hypochlorite.

B. AEROSOL SUITE

When pathogenic spores have been aerosolized or it is necessary to decontaminate the suite for any other reason, β-propiolactone (BPL) is used, employing the following procedure:

Six-hundred ml BPL (stored in refrigerator) are poured into the Challenger disseminator (Z and W Manufacturing Co., Wickliffe, Ohio) with extreme caution so as to prevent BPL from coming into contact with skin or clothing. The disseminator is sealed, placed in the exposure room, and plugged into the 110-V outlet which is controlled from the hallway. The disseminator switch is turned on, but the outlet switch remains off. An oscillating fan blowing into the anteroom is placed in the doorway between the exposure room and anteroom. At this time, the exhaust ducts in the anteroom and exposure room are sealed, creating a dead air space. The steam line is then turned on until the RH reaches 80%, as measured by the hygrometer: the steam valve is then turned off. The operator exits and immediately seals both doors to the suite with masking tape. The hall switch is turned on, activating the electric circuit. After 30-min dissemination time, the switch is turned off. The BPL is allowed to remain for a minimum of 2 hr, but preferably overnight. It is important that no one enter during this period. If entry must be made, only personnel having complete skin protection and wearing a gas mask may enter. After this time the area is air-washed for several hours, usually by opening the outside door (from the outside). The BPL disseminator is then carefully cleaned, the floor is hosed down and the exhaust ducts are opened. If the area is to remain in a decontaminated state, the interior doors to the hallway should be kept sealed and access accomplished by means of the outside door.

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